microPublication BIOLOGY

1/5/2021 - *Open Access*

unc-119 mutants have an increased fungal spore adhesion that is not rescued by *Cb-unc-119*

Shizue Omi[1] and Nathalie Pujol[1§]

[1]Aix Marseille Univ, INSERM, CNRS, CIML, Turing Centre for Living Systems, Marseille, France

[§]To whom correspondence should be addressed: pujol@ciml.univ-mrs.fr

Abstract

If the cuticle acts as a protective barrier against environmental insults, several pathogens have developed strategies that use it as a way to infect *C. elegans*. The fungus *Drechmeria coniospora* produces spores that attach to the cuticle, before hyphae invade the body. Mutants with an altered surface coat, the outermost layer of the cuticle, including *bus-2*, *bus-4*, *bus-12* and *bus-17* show increased adhesion of fungal spores (Rouger et al, 2014; Zugasti et al, 2016). We unexpectedly found that *D. coniospora* spores attach unusually densely around the mouth of *unc-119* mutants. Interestingly, this phenotype is not rescued by the *C. briggsae unc-119* construct that is conventionally used to rescue neuronal *unc-119* phenotypes.

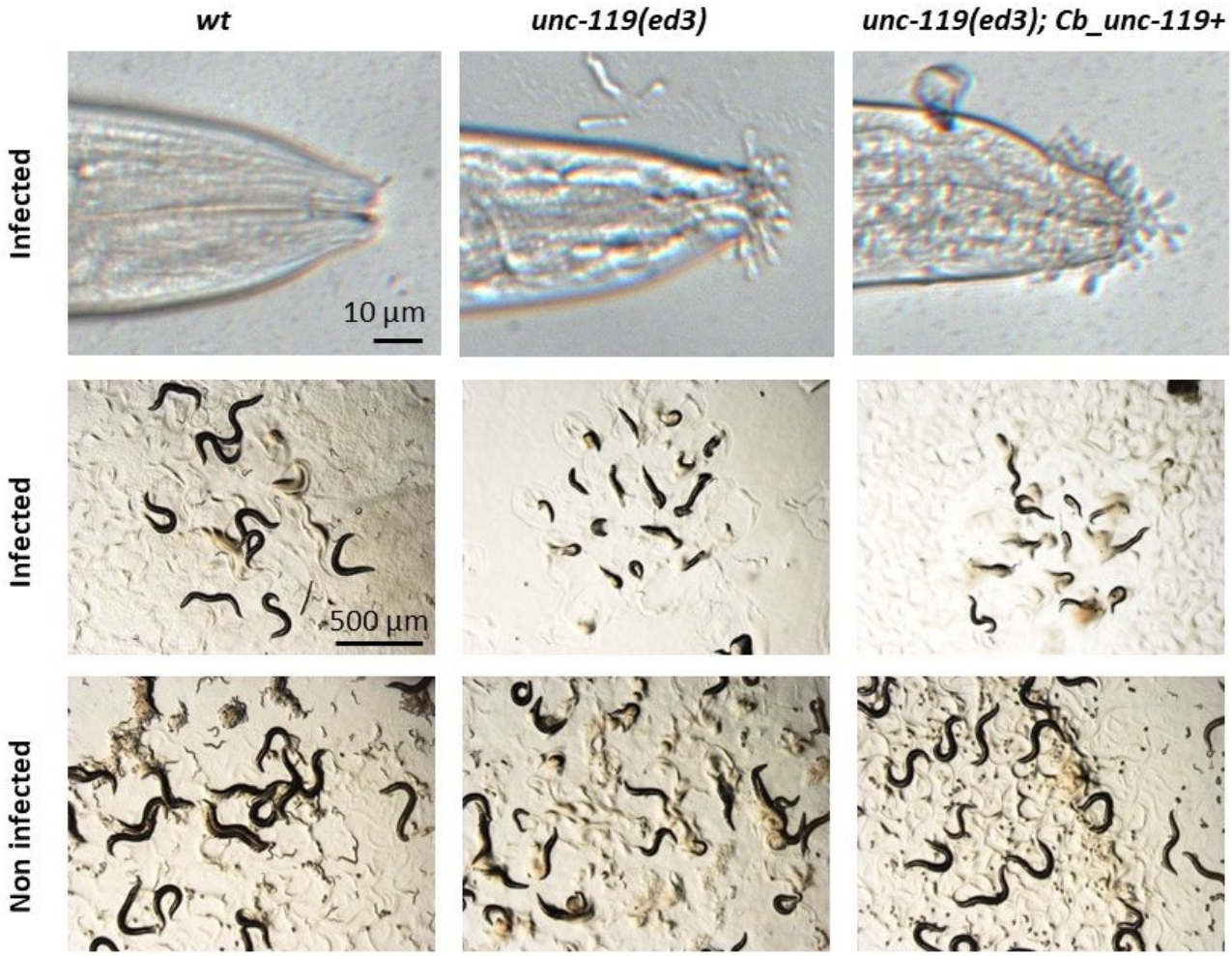

Figure 1. *unc-119* mutants are highly susceptible to infection by the fungus *Drechmeria coniospora*: After 24 h of infection by *D. coniospora*, *C. elegans* young adult worms were observed for the adhesion of spores at the mouth (upper row) or their overall morphology (middle row). Non-infected worms are shown for comparison (lower row). *unc-119* mutant worms carrying a wild-type copy of *Cb-unc-119* (*unc-119*(+), right column) or not (middle column) had an increased number of spores adhering to the mouth and an increased susceptibility to infection (reflected by a reduction in size), compared to wild-type worms (left column, *wt*).

Description

As part of our investigations of the interaction between *C. elegans* and *D. coniospora*, we made use of MosSCI strains, constructed in an *unc-119(ed3)* background (Frøkjær-Jensen *et al.*, 2008; Maduro, 2015). We noticed that a variety of

these strains exhibited a greatly increased susceptibility to infection. Upon further examination, we determined that this was due to an increase in the adhesion of fungal spores, most prominently at the tip of the head (Figure 1). The phenotype was not observed in a strain carrying a wild-type *C. elegans unc-119* rescuing construct in an *unc-119(e2498)* background. But the increased spore adhesion was visible in both the *unc-119(ed3)* and the *unc-119(tm4063)* background even in the presence of the standard *C. briggsae unc-119* rescuing construct. The phenotype was absent from these same transgenic strains in which *unc-119(ed3)* was eliminated by out-crossing (Table 1 below). *unc-119* function has been extensively analysed in the nervous system. Notably, some expression in the epidermis was reported recently (Lear *et al.*, 2018). While we have not determined the precise cause, since spore adhesion is a major determinant of infection progression (Zugasti *et al.*, 2016), such effects need to be taken into account when interpreting experiments involving any strain that has an *unc-119* allele in it, which has been often employed as selectable marker for transgenesis.

IG1629

strain	genotype	spore adhesion
N2	*wt*	normal
EG6699	*unc-119(ed3) III; ttTi5605 II*	increased
IG1604	*unc-119(ed3) III; frSi6[col-154p::CEBP-1::GFP::3'cebp-1, Cb-unc-119(+) ttTi5605] II*	increased
IG1633	*+; frSi6[col-154p::CEBP-1::GFP::3'cebp-1, Cb-unc-119(+) ttTi5605] II*	normal
IG1622	*unc-119(ed3) III; frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), Cb-unc-119(+) ttTi5605] II*	increased
IG1623	*+; frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), Cb-unc-119(+) ttTi5605] II*	normal
unc-119(ed3) III; frSi10[pNP150(F40H7.12p::GFP), Cb-unc-119(+) ttTi5605] II	increased	
AX6672	*unc-119(ed3) III; npr-1(ad609); ilcr-1(tm5866); [ilcr-1p::loxp::ILCR-1::let-858 3'UTR , Cb-unc-119(+) ttTi5605] II*	increased
OP533	*unc-119(tm4063) III; wgIs533[CEH-18::TY1::EGFP::3xFLAG, Cb-unc-119(+)]*	increased
JR667	*unc-119(e2498::Tc1) III; wIs51[SCMp::GFP, Ce-unc-119(+)] V*	normal

Methods

Request a detailed protocol

Eggs, prepared by the standard bleach method, were allowed to hatch in 50 mM NaCl in the absence of food at 25°C overnight. Synchronized L1 larvae were transferred to NGM agar plates spread with *E. coli* OP50 and cultured at 25°C until the L4 stage (40 h) before being exposed to fungal spores as previously described (Pujol *et al.*, 2001). Images were taken of worms mounted on a 2% agarose pad on a glass slide anesthetized with 0.25 mM levamisole, using a Zeiss AxioCam HR digital colour camera and AxioVision Rel. 4.6 software (Carl Zeiss AG).

Reagents

N2, EG6699 *unc-119(ed3); ttTi5605 II*, JR667 *unc-119(e2498::Tc1) III; wIs51[SCMp::GFP, Ce-unc-119(+)] V* and OP533 *unc-119(tm4063) III; wgIs533[CEH-18::TY1::EGFP::3xFLAG, Cb-unc-119(+)]* strains were provided by the CGC (*Caenorhabditis* Genetics Center), which is funded by NIH Office of Research Infrastructure Programs (P40 OD010440). In addition, the following strains were tested for spore adhesion: IG1604 *unc-119(ed3); frSi6[col-154p::CEBP-1::GFP::3'cebp-1, Cb-unc-119(+) ttTi5605] II* (Kim *et al.*, 2016), IG1622 *unc-119(ed3); frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), Cb-unc-119(+) ttTi5605] II*, IG1623 *frSi9[pNP151(col-62p::Lifeact::mKate_3'c-nmy), Cb-unc-119(+) ttTi5605] II* (Taffoni *et al.*, 2020), AX6672 *unc-119(ed3); npr-1(ad609); ilcr-1(tm5866); [ilcr-1p::loxp::ILCR-1::let-858 3'UTR, Cb-unc-119(+) ttTi5605] II* (Chen *et al.*, 2017), IG1633 *frSi6[pNP145(col-154p::CEBP-1::GFP::3'cebp-1), Cb-unc-119(+) ttTi5605] II* and IG1629 *unc-119(ed3); frSi10[pNP150(F40H7.12p::GFP), Cb-unc-119(+) ttTi5605] II*, this study. Both pNP145 and pNP150 were derived from pCFJ151 – ttTi5605_MCS, that was a gift from Erik Jorgensen (Addgene plasmid # 19330 ; http://n2t.net/addgene:19330 ; RRID:Addgene_19330) (Frøkjær-Jensen *et al.*, 2008).

Acknowledgments: We thank Jonathan Ewbank for his detailed and constructive comments.

References

Chen C, Itakura E, Nelson GM, Sheng M, Laurent P, Fenk LA, Butcher RA, Hegde RS, de Bono M. 2017. IL-17 is a neuromodulator of *Caenorhabditis elegans* sensory responses. Nature 542: 43-48. PMID: 28099418.

Frøkjaer-Jensen C, Davis MW, Hopkins CE, Newman BJ, Thummel JM, Olesen SP, Grunnet M, Jorgensen EM. 2008. Single-copy insertion of transgenes in *Caenorhabditis elegans*. Nat Genet 40: 1375-83. PMID: 18953339.

Kim KW, Thakur N, Piggott CA, Omi S, Polanowska J, Jin Y, Pujol N. 2016. Coordinated inhibition of C/EBP by Tribbles in multiple tissues is essential for *Caenorhabditis elegans* development. BMC Biol 14: 104. PMID: 27927209.

Lear, SK; Das, A; Goodman, MB (2018). Immunofluorescence reveals neuron-specific promoter activity in non-neuronal cells. microPublication Biology. 10.17912/8FDA-CK77.

Maduro MF. 2015. 20 Years of *unc-119* as a transgene marker. Worm 4: e1046031. PMID: 26430568.

Pujol N, Link EM, Liu LX, Kurz CL, Alloing G, Tan MW, Ray KP, Solari R, Johnson CD, Ewbank JJ. 2001. A reverse genetic analysis of components of the Toll signaling pathway in *Caenorhabditis elegans*. Curr Biol 11: 809-21. PMID: 11516642.

Rouger V, Bordet G, Couillault C, Monneret S, Mailfert S, Ewbank JJ, Pujol N, Marguet D. 2014. Independent synchronized control and visualization of interactions between living cells and organisms. Biophys J 106: 2096-104. PMID: 24853738.

Taffoni C, Omi S, Huber C, Mailfert S, Fallet M, Rupprecht JF, Ewbank JJ, Pujol N. 2020. Microtubule plus-end dynamics link wound repair to the innate immune response. Elife 9:e45047. PMID: 31995031.

Zugasti O, Thakur N, Belougne J, Squiban B, Kurz CL, Soulé J, Omi S, Tichit L, Pujol N, Ewbank JJ. 2016. A quantitative genome-wide RNAi screen in *C. elegans* for antifungal innate immunity genes. BMC Biol 14: 35. PMID: 27129311.

Funding: French National Research Agency (ANR-16-CE15-0001-01, ANR-16-CONV-0001) and institutional grants from CNRS, INSERM and Aix Marseille University to the CIML.

Author Contributions: Shizue Omi: Investigation. Nathalie Pujol: Supervision.

Reviewed By: Anonymous

History: Received December 14, 2020 **Revision received** December 29, 2020 **Accepted** December 30, 2020 **Published** January 5, 2021

Citation: Omi, S; Pujol, N (2021). *unc-119* mutants have an increased fungal spore adhesion that is not rescued by *Cb-unc-119*. microPublication Biology. https://doi.org/10.17912/micropub.biology.000344